BMC Pharmacology

Poster presentation

Possible identification of novel natriuretic peptide receptor phosphorylation sites by alanine/glutamate mutagenesis

Andrea R Yoder*[1], Kathryn A Barbieri[2], Jerid W Robinson[1] and Lincoln R Potter[1,2]

Address: [1]Department of Pharmacology, University of Minnesota, Minneapolis, MN 55455, USA and [2]Department of Biochemistry, Molecular Biology and Biophysics, University of Minnesota, Minneapolis, MN 55455, USA

Email: Andrea R Yoder* - yoder013@umn.edu

* Corresponding author

from 4th International Conference of cGMP Generators, Effectors and Therapeutic Implications
Regensburg, Germany. 19–21 June 2009

Published: 11 August 2009

BMC Pharmacology 2009, **9**(Suppl 1):P76 doi:10.1186/1471-2210-9-S1-P76

This abstract is available from: http://www.biomedcentral.com/1471-2210/9/S1/P76

Background

Natriuretic peptide receptors A (NPR-A) and B (NPR-B) are transmembrane guanylyl cyclases that regulate blood pressure, heart size and long bone growth. Unlike most cell surface receptors that are desensitized by direct phosphorylation, phosphorylation of natriuretic peptide receptors is essential for activation, and dephosphorylation causes their desensitization. While there are six and five known phosphorylation sites within NPR-A and NPR-B, respectively, studies in homologous sea urchin guanylyl cyclase receptors indicate the presence of 15–17 moles of phosphate per mole of receptor, suggesting additional natriuretic peptide receptor phosphorylation sites remain to be identified. The purpose of this study was to identify novel natriuretic peptide receptor phosphorylation sites by evaluating functional consequences of individual glutamate or alanine substitutions of candidate residues in order to mimic the effects of a phosphorylated or dephosphorylated residue, respectively.

Methods and results

Highly conserved serine and threonine residues among phosphorylated guanylyl cyclase receptors were mutated individually to either alanine or glutamic acid. The activity of these mutant receptors was measured by evaluating ligand-dependent guanylyl cyclase activities in membranes from transfected HEK293 cells. Since all known natriuretic peptide receptor phosphorylation sites are necessary for maximal receptor activation, initial analysis of putative sites assessed whether mutation glutamate and alanine substitutions resulted in receptors with more or less guanylyl cyclase activities, respectively.

Conclusion

Using a mutagenesis strategy to mimic phosphorylated and dephosphorylated residues, we identified novel conserved phosphorylation sites in NPR-A and NPR-B. Mutant receptors containing alanine and glutamate substitutions at these sites produced less active and more active receptors, respectively, consistent with phosphorylation being required for ANP-dependent activation and dephosphorylation mediating the desensitization of NPR-A. These findings are currently being verified using traditional tryptic phosphopeptide mapping of metabolically labelled receptors. This data will be discussed at the time of presentation.